Notes

Soft Drugs. 2. Soft Alkylating Compounds as Potential Antitumor Agents[1]

Nicholas Bodor*

Department of Medicinal Chemistry, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, Florida 32610

and James J. Kaminski

Inter$_x$ Research Corporation, Lawrence, Kansas 66044. *Received August 1, 1979*

A class of soft alkylating compounds as potential anticancer agents was developed. The first examples include α-halo esters of various carboxylic acids. A new method for quantitative evaluation of the alkylating reactivity was developed, using a competitive alkylation reaction, followed by NMR analysis of the reaction mixture. The method is sensitive and reproducible. One of the two selected soft alkylating agents, chloromethyl hexanoate, was found to have anticancer activity.

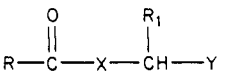
The development of target-specific drugs is perhaps more important in cancer therapy than in any other fields. Soft anticancer agents would be one of the most attractive ways to achieve this objective, particularly if combined with appropriate chemical (i.e., site-specific carrier molecules) or therapeutic (i.e., regional perfusion or intraarterial infusion) delivery approaches. "Soft drugs" can be defined[2] as biologically active chemical compounds (drugs) which are characterized by a predictable in vivo destruction (metabolism) to nontoxic moieties, after they achieve their therapeutic role. The metabolic disposition of the soft drugs takes place with a *controllable rate* in a *predictable manner*. An ideal soft anticancer agent should thus have low systemic toxicity in lieu of its controllable and predictable cleavage to nonactive, nontoxic species.

During the recent development of soft quaternary salts,[1] a number of "soft" alkylating agents were prepared. While the soft quaternary salts are "soft analogues", the soft alkylating agents belong to a different class of drugs, i.e., activated soft compounds. Drugs in this class are not analogues of known drugs. They are obtained by introducing activating groups in essentially nontoxic compounds. The activated forms revert to the basic nontoxic moiety or fall further apart to nontoxic parts during the process or performing their therapeutic role. For example, the potent germicidal *N*-halo compounds based on amino acid derivatives or amino alcohol esters[3-5] revert to the basic amino compound while transferring their positive chlorine.

The present soft alkylating agents can formally be derived from simple alkanol esters of aliphatic or aromatic acids via introducing a halogen to the α carbon of the alcohol portion. These α-halo esters are relatively weak alkylating agents; thus, it was assumed that many of the alkylating compounds could have antitumor activity. On the other hand, as activated esters, all these compounds are subject to hydrolytic (enzymatic and/or chemical) cleavage–deactivation; hence, their overall toxicity should be lower than that of the conventional alkylating agents, which can be deactivated primarily through an alkylating process.

The present work describes the preliminary efforts in this direction and the design, reactivity, and activity of these soft alkylating agents.

Chemistry. The general structure of the soft alkylating agents is given by by **1**. The present work describes



1, R = alkyl, aryl, etc.; R_1 = H or R; X = NH, O, or S; Y = Cl, Br, I, CH_3OSO_2, etc.

studies on the compounds where R and R_1 are limited to a few simple alkyl and aryl groups, X is O, and Y is either Cl or Br, as shown in Table I.

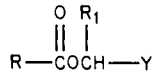
All the α-halo esters **1a–h** can easily be prepared by the condensation of equimolar amounts of an acyl halide and the corresponding R_1CHO aldehyde, using the methods previously described.[1,6] Compounds **1a–h**, as "activated esters", are clearly subject to chemical and/or enzymatic ester cleavage, the rate of which can be controlled by the functions R, R_1, X, and Y.

Relative Reactivity of the Alkylating Agents. The activity–toxicity characteristics of the various alkylating agents depend on a number of factors, such as transport properties, partition coefficients, etc., and also on their intrinsic alkylating activities. No method is, however, available to determine the relative alkylating power of the various alkylating agents. The known analytical methods, based on colorimetry using (4-nitrobenzyl)pyridine[7] or other related reagents (4-pyridinecarboxyaldehyde 4-nitrophenylhydrazone[8] and others), are quite suitable for

(1) For Part 1 of this series, see N. Bodor, J. J. Kaminski, and S. Selk, *J. Med. Chem.*, **23**, under Articles in this issue (1980).
(2) N. Bodor, in "Design of Bio-Pharmaceutical Properties through Prodrugs and Analogs", E. B. Roche, Ed., Academy of Pharmaceutical Sciences, Washington, D.C., 1977, p 98.
(3) N. Bodor, J. J. Kaminski, S. D. Worley, R. J. Colton, T. H. Lee, and J. W. Rabalais, *J. Pharm. Sci.*, **63**, 1387 (1974).
(4) J. J. Kaminski, N. Bodor, and T. Higuchi, *J. Pharm. Sci.*, **65**, 553 (1976); *ibid.*, **65**, 1733 (1976).
(5) J. J. Kaminski, M. M. Huycke, S. H. Selk, N. Bodor, and T. Higuchi, *J. Pharm. Sci.*, **65**, 1737 (1976).
(6) (a) R. Adams and E. H. Vollweiler, *J. Am. Chem. Soc.*, **40**, 1732 (1978); (b) L. H. Ulich and R. Adams, *ibid.*, **43**, 660 (1921).
(7) J. Epstein, R. W. Rosenthal, and R. J. Ess, *Anal. Chem.*, **27**, 1435 (1955).
(8) E. Sawicki, D. F. Bender, T. R. Hauser, R. M. Wilson, Jr., and J. E. Meeker, *Anal. Chem.*, **35**, 1479 (1963).

0022-2623/80/1823-0566$01.00/0

Table I. Characteristics of the Soft Alkylating Agents[a]

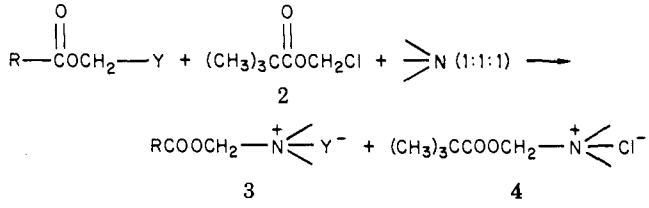


1

no.	R	R_1	Y	purifn method[b]	bp, °C (mmHg)	formula	anal.
1a	$CH_3(CH_2)_4$-	H	Cl	A	54-57 (0.5)	$C_7H_{13}ClO_2$	C, H
1b		H	Cl	A	72-74 (0.6)	$C_8H_{13}ClO_2$	C, H
1c		H	Cl	A	57-59 (0.3)	$C_8H_{11}ClO_2$	C, H
1d	$C_6H_5CH_2$	H	Cl	A	132-134 (14)	$C_9H_9ClO_2$	*c*
1e		H	Cl	B		$C_{14}H_{17}ClO_2$	C, H
1f	C_6H_5-	H	Cl	A	114-115 (8)	$C_8H_7ClO_2$	*d*
1g	C_6H_5-	H	Br	A	62-65 (0.3)	$C_8H_7BrO_2$	*d*
1h	$CH_3(CH_2)_4$-	CH_3	Cl	B		$C_8H_{15}ClO_2$	C, H

[a] Synthesized according to the general method described under Experimental Section, where typical spectral data for 1a and 1f can also be found. [b] A = distillation; B = chromatography on Florisil. [c] See ref 6a. [d] See ref 6b.

Scheme I

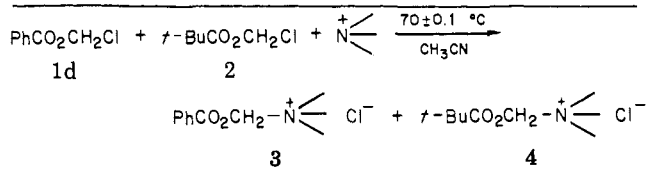


quantitative determination of the more reactive alkylating agents, or even of *N*-mustards,[9] but they are not sensitive enough to separate closely related agents by their relative reactivity. Our attempts to use these reagents and determine the relative rates of formation of the corresponding quaternary salts with the soft alkylating agents have failed, since, due to the low reactivities of these reagents as well as that of the alkylating agents studied, harsh conditions for the reaction had to be used, where accurate determination of the rate constants was impossible.

It appears that a competitive alkylation would provide a good method for determining the relative alkylating activities. Thus, a 1:1:1 molar mixture of (1) a standard alkylating agent, of (2) the unknown alkylating agent, and (3) a suitable amine should provide a reaction mixture in which the ratio of the relative amounts of the two quaternary salts quantitatively describes their relative reactivities. In this way, a scale of alkylating reactivity can be constructed. The reaction mixture composition can easily be analyzed by NMR, comparing the sharp singlet resonance signals corresponding to the $\geq N^+CH_2O$-C(=O)-R methylene, at extended sweep widths. The easily available and stable soft alkylating agent chloromethyl pivalate (**2**) was chosen as the standard in the series studied. The method, thus, can be described as shown in Scheme I.

The ratio **3**/**4** is the relative alkylating reactivity (RAR) of 1. The conditions used were acetonitrile as a solvent and heating the reaction mixture to 70 °C for 1 h. First, a series of amines was compared using a selected alkylating agent [chloromethyl benzoate (**1f**), together with **2**] and triethylamine, 3-acetoxyquinuclidine (**5**), pyridine, and *N*-methylimidazole as the amine counterpart. As one can see in Table II, the nature of the amine does not affect much the ratio RAR, except the *N*-methylimidazole. Due to the molecular rigidity, reproducibility, and easy handling, we have selected 3-acetoxyquinuclidine (**5**) as the standard amine.

Table II. Effect of Amines on the Relative Alkylating Reactivity



amine	product composition, %[a] 3	4	RAR ratio: 3/4
Et_3N	42	58	0.72
	42	58	0.72
	41	59	0.69
	40	60	0.67
	40	60	0.67
	48	52	0.92
	45	55	0.82

[a] The percent product composition values are the average of two separate experiments found to be reproducible within ±0.5%. Multiple integration (5 determinations) of the appropriate resonance signal was used.

The soft quaternary salts given in Table III in all cases were separately prepared and identified prior to the determination of RAR.

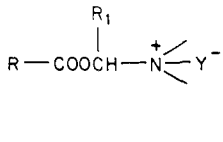
For comparison, benzyl chloride (**6**) was also included, leading to the corresponding hard quaternary salt (**7**) when reacted with **5**.

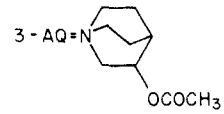
The results of the studies are included in Table IV. It can be seen that RAR varies in a wide range, from 0.5 to about 13.

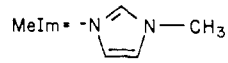
As expected, benzyl chloride (**6**) is a much more reactive alkylating agent than the analogous soft alkylating **1f**. It is also evident that the nature of the halogen is very important, as **1g** is 20 times more reactive than **1f**. The steric bulk around the α carbon seems to have the largest effect

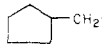
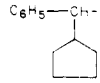
(9) O. M. Friedman and E. Boger, *Anal. Chem.*, 33, 906 (1961).

Table III. Soft Quaternary Salts Characterized and Studied[a]



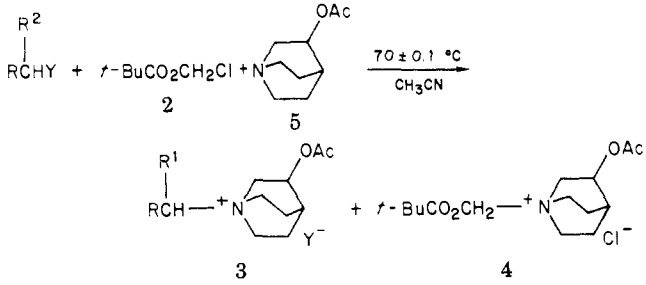




no.[b]	R	R_1	N⊲[c]	mp, °C	formula	anal.
3a	$CH_3(CH_2)_4$-	H	3-AQ	118-120	$C_{16}H_{28}ClNO_4$	C, H, N
3b		H	3-AQ	145-148°	$C_{17}H_{28}ClNO_4$	C, H, N
3d	C_6H_5-CH_2-	H	3-AQ	77-80	$C_{18}H_{24}ClNO_4$	C, H, N
3e		H	3-AQ	171-174	$C_{22}H_{32}ClNO_4$	C, H, N
3f	C_6H_5	H	TEA	173-175[d]	$C_{14}H_{22}ClNO_2$	C, H, N
3g	C_6H_5	H	3-AQ	188-191[d]	$C_{17}H_{22}ClNO_4$	C, H, N
3h	C_6H_5	H	Py	197-199[d]	$C_{13}H_{12}ClNO_2$	C, H, N
3i	C_6H_5	H	MeIm	149-152[d]	$C_{12}H_{13}ClN_2O_2$	C, H, N
3j	C_6H_5	H	3-AQ	190-193	$C_{17}H_{22}BrNO_4$	C, H, N
4a	$(CH_3)_3C$-	H	TEA	197-201[d]	$C_{12}H_{26}ClNO_2$	C, H, N
4b	$(CH_3)_3C$-	H	3-AQ	184-188[d]	$C_{15}H_{26}ClNO_4$	C, H, N
4c	$(CH_3)_3C$-	H	Py	123-125	$C_{11}H_{16}ClNO_2$	C, H, N
4d	$(CH_3)_3C$-	H	MeIm	139-141	$C_{10}H_{17}ClN_2O_2$	C, H, N

[a] General synthetic method together with characteristic spectral data are given under Experimental Section. [b] Y is Cl in all cases, except 3j, where Y = Br. [c] Py = pyridine; TEA = $N(CH_2CH_3)_3$. [d] Decomposition.

Table IV. Relative Alkylating Reactivity of Selected Soft Alkylating Agents



no.	product composition, %[a] 3	4	RAR ratio: 3/4
6[b]	79	21	3.76
1a	35	65	0.54
1b	40	60	0.67
1c	44	56	0.79
1d	73	27	2.70
1e	48	52	0.92
1f	40	60	0.67
1g	93	7	13.29
1h		>99	0

[a] See footnote *a*, Table I. [b] Benzyl chloride (6) was included for comparison.

on the RAR; thus, 1h is less reactive than the homologous 1a by orders of magnitude.

Biology. For preliminary studies, the two hexanoyl homologues 1a and 1h were selected and submitted to the NCI for testing. It is quite encouraging that, while 1h, having very low RAR, was inactive, the typical, simple soft alkylating agent 1a (NSC 281814D) was found active in the P388 lymphocytic leukemia (ILS > 125%), which was confirmed.

Discussion

The class of the soft alkylating agents described in the present work is a good example of the design of new, soft drugs. The structural possibilities based on the general formula are practically infinite, but logical selections of certain classes of acids should lead to the development of active, site-specific or selective, soft antitumor agents. The mechanism of action is not known: Is the alkylation itself the formaldehyde formed at the reaction site[10] or is another mechanism responsible for the antitumor activity?

There is no reason to believe that the simple chloromethyl hexanoate (**1a**) is the optimum structure. The basic acid, or the corresponding hexanoates, do not have any specific biological role. The compound **1a** has very low water solubility. It is, however interesting to see such simple molecules having anticancer activity; thus, studies of the various possible α-halo esters and related compounds are well warranted.

The competitive alkylation method developed seems to be a very useful tool for preliminary screening and ranking of the alkylating agents. A combination of the RAR and partition coefficients (transport properties), together with enzymatic hydrolysis studies of the various esters, should lead to the optimized anticancer drugs of this type.

The current studies involve a number of directions: optimization of the carrier portion by judicious selection of the basic acid structure; comparison of the ester, amide, and thioester type derivatives and investigation of the importance of two alkylating groups, as suggested for the anticancer activity of "hard" alkylating agents[11] (for example, by structures like $ClCH_2OCOC(R_2)HCOOCH_2Cl$); comparison of chemical activities (RAR) and toxicities to *N*-mustards; SAR concerning the aldehyde portion and the halogen, etc. Activity studies on 1a will be continued in the meantime at the NCI.

Experimental Section

Synthesis. Chloromethyl pivalate (**2**) and benzyl chloride (**6**) were obtained from Aldrich Chemical Co.

General Method for the Preparation of 1a-h. Equimolar amounts of the corresponding RCOY aryl halide and paraformaldehyde (or paraldehyde) were heated at 90–100 °C for 4 h. The

(10) W. Ross, "Biological Alkylating Agents", Butterworths, London, 1962.
(11) A. Haddow, G. Kon, and W. Ross, *Nature (London)*, **162**, 284 (1948).

obtained α-halo esters were purified by distillation or chromatography, as shown in Table I. Characteristic spectral data for selected typical compounds are as follows.

Chloromethyl *n*-hexanoate (1a): bp 54–57 °C (0.5 mm); IR (neat) 2970, 2930, 1750, 1435, 1135, 1100, 1040 and 720 cm^{-1}; NMR ($CDCl_3$) δ 5.67 (s, 2 H), 2.5 (t, 2 H), 0.8–2.0 (8 H).

Chloromethyl benzoate (1f): IR (neat) 3060, 2990, 1730, 1590, 1490, 1450, 1340, 1300, 1250, 1080, 800, 720 cm^{-1}; NMR ($CDCl_3$) δ 7.0–8.2 (m, 5 H), 5.97 (s, 2 H).

α-Chloroethyl *n*-hexanoate (1h): IR (neat) 2960, 2920, 1750, 1450, 1380, 1270, 1230, 1160, 1050, 1030, 950, 670 cm^{-1}; NMR ($CDCl_3$) δ 6.50 (q, 1 H), 2.33 (t, 2 H), 1.8 (d, 3 H), 0.7–1.7 (9 H).

Soft quaternary salts **3a–k** and **4a–d** were prepared by mixing equimolar amounts of the corresponding **1** or **2** with the amine in acetonitrile at 70 °C. The product was isolated by crystallization. The yields were usually close to quantitative. Characteristic spectral data are as follows.

1-[(*n*-Hexanoyloxy)methyl]-3-acetoxyquinuclidinium chloride (3a): IR ($CHCl_3$) 2950, 1750, 1460, 1365, 1100, 900 cm^{-1}; NMR (D_2O) δ 5.17 (s, 2 H), 3.2–4.4 (9 H), 1.2–3.2 (11 H), 2.1 (s, 3 H), 0.9 (t, 3 H).

1-[(Phenylacetoxy]-3-acetoxyquinuclidinium chloride (3): IR ($CHCl_3$) 2960, 1740, 1460, 1370, 1210, 1120, 1080, 930 cm^{-1}; NMR (D_2O) δ 7.4 (s, 5 H), 5.23 (s, 2 H), 3.90 (s, 2 H), 3.0–4.0 (7 H), 1.8–3.0 (5 H), 2.1 (s, 3 H).

[(Benzoyloxy)methyl]triethylammonium chloride (3f): IR (KBr) 3010, 2990, 1720, 1595, 1450, 1260, 1180, 1110, 1080, 805, 720 cm^{-1}; NMR (D_2O) δ 7.4–8.4 (5 H), 5.50 (s, 2 H), 3.5 (q, 6 H), 1.47 (t, 9 H).

1-[(Benzoyloxy)methyl]-3-acetoxyquinuclidinium bromide (3j): IR (KBr) 2975, 1720, 1600, 1450, 1370, 1240, 1110, 1030, 920, 720 cm^{-1}; NMR (D_2O) δ 7.2–8.2 (5 H), 5.47 (s, 2 H), 2.0–4.3 (12 H), 2.2 (s, 3 H).

1-[(Pivaloyloxy)methyl]-3-acetoxyquinuclidinium chloride (4b): IR (KBr) 2980, 1710, 1370, 1250, 1150, 1110 cm^{-1}; NMR (D_2O) δ 5.23 (s, 2 H), 3.2–4.2 (6 H), 1.9–3.0 (6 H), 2.2 (s, 3 H), 1.3 (s, 9 H).

3-Acetoxyquinuclidine (5). 3-Quinuclidinol, 25.4 g (0.02 mol), was dissolved at 0 °C in 87 mL (1.08 mol) of pyridine and 31 mL (0.33 mol) of acetic anhydride was added. The solution was heated under reflux for 2 h and the excess pyridine was removed by distillation in vacuo. The residue was dissolved in chloroform and washed with saturated potassium carbonate. The chloroform solution was dried over anhydrous sodium sulfate. Following filtration, the chloroform was removed under reduced pressure to afford a brown-colored liquid. Vacuum distillation of this material gave 23.6 g (0.14 mol, 70%) of **5**: mp 34–36 °C; bp 76–78 °C (0.8 mm); IR (neat) 2950, 2920, 1700, 1435, 1350, 1230, 1020, 780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.8 (m, 1 H), 2.4–3.6 (6 H), 2.10 (s, 3 H) and 1.1–2.0 (5 H). Anal. ($C_9H_{15}NO_2$) C, H, N.

1-Benzyl-3-acetoxyquinuclidinium chloride (7): mp 205–207 °C; IR (KBr) 2980, 1730, 1365, 1240, 1025, 770, 710 cm^{-1}; ^1H NMR (D_2O) δ 7.6 (s, 5 H), 5.10 (m, 1 H), 4.47 (s, 2 H), 3.2–4.0 (6 H), 2.1 (s, 3 H), 1.8–2.4 (5 H). Anal. ($C_{16}H_{22}ClNO_2$) C, H, N.

Table V. Activity of a Soft Alkylating Agent 1a in P388 Lymphocytic Leukemia[a]

dose, mg/kg	no. of animals	% ILS[b]
400	5	109
200	6	127[c]
100	6	120
50	6	107

[a] One daily dose; total number of doses = 9. Vehicle: saline, administration by ip injection. 1a is NSC 281814D. [b] Percent increase in median survival time as compared to the control group. [c] Activity confirmed by second test.

Competitive Alkylations. a. Determination of Amine Selectivity. To an acetonitrile solution (9 mL) containing 189.0 mg (1.26 mmol) of chloromethyl pivalate (**2**) and 214.2 mg (1.26 mmol) of chloromethyl benzoate (**1f**) was added 212.9 mg (1.25 mmol) of 3-acetoxyquinuclidine (**5**) dissolved in 6 mL of acetonitrile. The solution was heated at 70 ± 0.1 °C for 1 h. The acetonitrile was removed under reduced pressure and the residue obtained was dried in vacuo over anhydrous calcium sulfate. The residue was dissolved in D_2O–acetone-d_6 (~1:2, v/v). The composition of the isolated product mixture was determined by multiple integration (five determinations) of the $\geqslant N^+CH_2O_2CR$ resonance signal of the products at expanded sweep widths (250 Hz). The corresponding very sharp singlet signals were well separated and could easily be integrated.

Using the procedure described for determining the selectivity of 3-acetoxyquinuclidine (**5**) relative to chloromethyl pivalate (**2**) and chloromethyl benzoate (**1f**), triethylamine, pyridine, and 1-methylimidazole were also investigated. The results are shown in Table II.

b. Determination of the Relative Alkylating Reactivity (RAR). The procedure described above was used, mixing 1.26 mmol of **2** and 1.26 mmol of **1a–h** and **6**, respectively, and 1.26 mmol of **5** in acetonitrile. The results are shown in Table IV.

Biology. The screening of **1a** and **1h** were performed by the Drug Evaluation Branch of the NCI. The results are given in Table V.

Synthesis of Some New S-Alkylated Derivatives of 5-Mercapto-2′-deoxyuridine as Potential Antiviral Agents

Frank J. Dinan*[1] and Thomas J. Bardos

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Amherst, New York 14260. *Received August 6, 1979*

A series of S-alkylated derivatives of 5-mercapto-2′-deoxyuridine have been prepared by alkylation of the preformed nucleoside. Two of these compounds, the *S*-propargyl and *S*-allyl derivatives, have shown significant antiviral activity against *Herpes simplex* type 1 in HeLa TK^- cells but appear to be less effective in this assay system than some previously reported 5-substituted 2′-deoxyuridines.

Since the discovery of the anti-herpes activity of 5-iodo-2′-deoxyuridine,[2,3] the first therapeutically employed antiviral nucleoside, a number of other 5-substituted 2′-deoxyuridines have been found to possess significant activity against the *Herpes simplex* virus. These include the clinically effective 5-(trifluoromethyl)[4,5] and 5-ethyl[6] derivatives, as well as the mostly in vitro tested 5-bromo-,[7]

(1) On leave from the Department of Chemistry, Canisius College, Buffalo, N.Y.
(2) W. H. Prusoff, M. S. Chen, P. H. Fischer, T.-S. Lin, G. T. Shian, and R. F. Schinazi, *Adv. Ophthalmol.*, **38**, 3–16 (1979).
(3) J. Sugar and H. E. Kaufman in "Selective Inhibitors of Viral Functions", W. A. Carter, Ed., CRC Press, Cleveland, Ohio, 1973, pp 295–311.
(4) C. Heidelberg, *Ann. N.Y. Acad. Sci.*, **255**, 317 (1975).
(5) F. Bigar, *Adv. Ophthalmol.*, **38**, 105–109 (1979).
(6) K.-L. Elze, *Adv. Ophthalmol.*, **38**, 134–139 (1979).

0022-2623/80/1823-0569$01.00/0